Structure and Dynamics of 6-Hydroxymethyl-7,8-dihydropterin Pyrophosphokinase

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Introduction: Folates are essential for life. Unlike mammals, most microorganisms must synthesize folates *de novo*. 6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) catalyzes pyrophosphoryl transfer from ATP to 6-hydroxymethyl-7,8-dihydropterin (HP), the first reaction in folate pathway, and therefore, is an ideal target for developing novel antimicrobial agents. Because of its small size and high thermal stability, *E. coli* HPPK is also an excellent model enzyme for studying the mechanisms of enzymatic pyrophosphoryl transfer.

Methods and Materials: Single crystal X-ray diffraction.

Results: We have determined the crystal structures of HPPK in the unligated form and in complex with HP, two Mg^{2+} ions, and AMPCPP (an ATP analog that inhibits the enzymatic reaction). Comparison of the two crystal structures reveals dramatic conformational changes of three flexible loops and many side chains and possible roles of the active site residues.

Conclusions: The binding of HP and MgAMPCPP induces dramatic conformational changes in HPPK. If the apo-protein looks like a half-closed right hand, the ternary complex appears to be a tightly closed fist. The most significant conformational differences reside in three flexible loops. In apo-HPPK, Loop-1 assumes two conformations with almost equal probabilities, whereas in the ternary complex, it has one well-defined conformation. Loops 2 and 3 move in to close the catalytic center.

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